

Currently Pending Claims

1. (Amended) A method for establishing an association between a first gene and a selected phenotype in a cell, the method comprising the steps of:

(i) selecting a first gene, wherein the first gene is not operably linked to heterologous sequences,

(ii) selecting a second gene, wherein the second gene is different from the first gene and wherein the second gene is not operably linked to heterologous sequences;

(iii) providing a first zinc finger protein that binds to a first target site in the first gene and modulates expression of the first gene;

(iv) providing a second zinc finger protein that binds to a second target site in the second gene and modulates expression of the second gene;

(v) culturing a first cell under conditions where the first zinc finger protein contacts the first gene;

(vi) culturing a second cell under conditions where the second zinc finger protein contacts the second gene; and

(vii) assaying the first and second cells for the selected phenotype, wherein a change in the selected phenotype in the first cell as compared to the second cell indicates an association between the first gene and the selected phenotype.

2. (Amended) The method of claim 1, further comprising providing a third zinc finger protein that binds to a third target site in the first gene, wherein the third target site is different than the first target site.

3. Canceled.

4. Canceled.

5. (Amended) The method of claim 1, wherein the first gene is partially encoded by an EST.

6. (Amended) The method of claim 1, wherein the first and second cells are derived from the same cell type.

7. Canceled.

8. (Amended) The method of claim 1, wherein the first and the second genes are endogenous cellular genes.

9. (Amended) The method of claim 1, wherein the modulation is repression.

10. (Amended) The method of claim 1, wherein the modulation is activation.

11. The method of claim 1, wherein the zinc finger proteins are fusion proteins comprising a regulatory domain.

12. (Amended) The method of claim 11, wherein the function of the regulatory domain is under small molecule control.

13. The method of claim 11, wherein the zinc finger proteins are fusion proteins comprising at least two regulatory domains.

14. The method of claim 1, wherein the cell is selected from the group consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

15. The method of claim 11, wherein the cell is a mammalian cell.

16. The method of claim 12, wherein the cell is human cell.

17. The method of claim 1, wherein expression is activation of gene expression that prevents repression of gene expression.

18. The method of claim 1, wherein the modulation of expression is inhibition of gene expression that prevents gene activation.

19. The method of claim 11, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, a methyl transferase, a transcriptional activator, a histone acetyltransferase, and histone deacetylase.

20. (Amended) The method of claim 1, wherein the first zinc finger protein is encoded by a first expression vector comprising a first zinc finger protein-encoding nucleic acid operably linked to a first promoter, and step (v) further comprises administering the first expression vector to the first cell.

21. (Amended) The method of claim 20, wherein the second zinc finger protein is encoded by a second expression vector comprising a second zinc finger protein-encoding nucleic acid operably linked to a second promoter, and step (vi) further comprises administering the second expression vector to the second cell.

22. (Amended) The method of claim 20, wherein the first expression vector further comprises a second zinc finger protein-encoding nucleic acid operably linked to a promoter.

23. (Amended) The method of claim 22, wherein the first and second zinc finger protein-encoding nucleic acids are operably linked to the same promoter.

24. The method of claim 20, wherein the expression vector is a viral vector.
25. The method of claim 24, wherein the expression vector is a retroviral expression vector, an adenoviral expression vector, or an AAV expression vector.
26. The method of claim 20, wherein the first and second zinc finger proteins are encoded by a nucleic acid operably linked to an inducible promoter.
27. The method of claim 1, wherein the cell comprises less than about 1.5×10^6 copies of the zinc finger protein.
28. (Amended) The method of claim 1, wherein expression of one or more of the zinc finger proteins is induced by administration of an exogenous agent.
29. (Amended) The method of claim 22, wherein the first and second zinc finger protein-encoding nucleic acids are operably linked to different promoters.
30. (Amended) The method of claim 20, wherein expression of the first zinc finger protein is controlled by a small molecule.
31. (Amended) The method of claim 21, wherein expression of the second zinc finger protein is controlled by a small molecule.
32. (Amended) The method of claim 22, wherein expression of both the first and second zinc finger proteins are controlled by a small molecule.
33. (Amended) The method of claim 32, wherein expression of the first zinc finger protein and expression of the second zinc finger protein are controlled by different small molecules.
- 34 through 86. Canceled.
87. A method for determining the association between a gene and a phenotype of a cell, the method comprising the steps of:
- (i) providing first, second and third cells,
 - (ii) contacting the first cell with a first zinc finger protein that binds to a first target site in the gene and activates expression of the gene;
 - (iii) contacting the second cell with a second zinc finger protein that binds to a second target site in the gene and represses expression of the gene;
 - (iv) assaying the first, second and third cells for the selected phenotype; and
 - (v) comparing the phenotypes exhibited by the first, second and third cells, wherein if the first or second cell exhibits a different phenotype than the third cell, the gene is associated with

the phenotype.

88. The method of claim 87, wherein first and second target sites are different.
89. The method of claim 87, wherein first and second target sites are the same.
90. The method of claim 87, wherein the first and second zinc finger proteins are each fused to a regulatory domain.
91. The method of claim 90, wherein the regulatory domains are the same.
92. The method of claim 91, wherein function of the regulatory domain is dependent on a small molecule.
93. The method of claim 87, further comprising the step of exposing the first, second and third cells to at least one selected stimulus prior to assaying for a selected phenotype.
94. The method of claim 93, wherein the phenotype assayed is a change in cell physiology.
95. The method of claim 93, wherein the selected stimulus is serum starvation, growth factor depletion or growth factor stimulation.
96. The method of claim 95, wherein the phenotype assayed is cell proliferation.
97. The method of claim 96, wherein the phenotype assayed is a change in cell cycling.
98. The method of claim 93, wherein the selected stimulus is stress.
99. The method of claim 98, wherein the stress is selected from the group consisting of reducing agents, oxidizing agents, mutagens, DNA synthesis inhibitors, DNA damaging agents, heat shock, cold shock, hypoxia, and altered pressure.
100. The method of claim 99, wherein the DNA damaging agent is a chemical.
101. The method of claim 99, wherein the DNA damaging agent is irradiation.
102. The method of claim 98, wherein the phenotype assayed is a change in cell metabolism.
103. The method of claim 102, wherein the change in cell metabolism is assayed using a transformation assay.
104. The method of claim 93, wherein the selected stimulus is exposure to a pathogen.

105. The method of claim 104, wherein the pathogen is a bacterium.
106. The method of claim 104, wherein the pathogen is a virus.
107. The method of claim 104, wherein the pathogen is a unicellular eukaryote.
108. The method of claim 93, wherein the selected stimulus is treatment with a compound.
109. The method of claim 87, wherein the first, second and third cells further comprise an exogenous nucleic acid.
110. The method of claim 109, wherein the exogenous nucleic acid encodes a polypeptide.
111. The method of claim 110, wherein the polypeptide is an endogenous polypeptide.
112. The method of claim 110, wherein the polypeptide is a mutant form of an endogenous polypeptide.
113. The method of claim 87, wherein the association between the gene and the phenotype indicates a biological function of the gene.